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=> index bioscience
FILE 'DRUGMONOG' ACCESS NOT AUTHORIZED
COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                         ENTRY    SESSION
FULL ESTIMATED COST                                       0.21       0.21

INDEX 'ADISCTI, ADISINSIGHT, ADISNEWS, AGRICOLA, ANABSTR, ANTE, AQUALINE,
       AQUASCI, BIOENG, BIOSIS, BIOTECHABS, BIOTECHDS, BIOTECHNO, CABA, CAPLUS,
       CEABA-VTB, CIN, CONFSCI, CROPB, CROPU, DDFB, DDFU, DGENE, DISSABS, DRUGB,
       DRUGMONOG2, DRUGU, EMBAL, EMBASE, ...' ENTERED AT 15:44:21 ON 18 JAN 2006

70 FILES IN THE FILE LIST IN STNINDEX

Enter SET DETAIL ON to see search term postings or to view
search error messages that display as 0* with SET DETAIL OFF.

=> fluorescent and polypeptide oligomerize
  23 FILES SEARCHED...
  62 FILES SEARCHED...
           3   FILE USPATFULL
           1   FILE USPAT2

   2 FILES HAVE ONE OR MORE ANSWERS,   70 FILES SEARCHED IN STNINDEX

L1   QUE FLUORESCENT AND POLYPEPTIDE OLIGOMERIZE

=> fluorescent and polypeptide and oligomerize
           1   FILE BIOSIS
           5   FILE BIOTECHABS
           5   FILE BIOTECHDS
           2   FILE CAPLUS
          93   FILE DGENE
  23 FILES SEARCHED...
           1   FILE EMBASE
           1   FILE ESBIOBASE
          10   FILE IFIPAT
           1   FILE LIFESCI
           4   FILE MEDLINE
           1   FILE SCISEARCH
           1   FILE TOXCENTER
         279   FILE USPATFULL
          34   FILE USPAT2
           6   FILE WPIDS
  68 FILES SEARCHED...
           6   FILE WPINDEX

  16 FILES HAVE ONE OR MORE ANSWERS,   70 FILES SEARCHED IN STNINDEX

L2   QUE FLUORESCENT AND POLYPEPTIDE AND OLIGOMERIZE

=> d rank
F1          279   USPATFULL
F2           93   DGENE
F3           34   USPAT2
F4           10   IFIPAT
F5            6   WPIDS
F6            6   WPINDEX
F7            5   BIOTECHABS
F8            5   BIOTECHDS
F9            4   MEDLINE
F10           2   CAPLUS
F11           1   BIOSIS
F12           1   EMBASE
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F13 1 ESBIOBASE
F14 1 LIFESCI
F15 1 SCISEARCH
F16 1 TOXCENTER

=> file biotechabs medline caplus biosis

COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	3.66	3.87

FILE 'BIOTECHABS' ACCESS NOT AUTHORIZED

FILE 'MEDLINE' ENTERED AT 15:48:10 ON 18 JAN 2006

FILE 'CAPLUS' ENTERED AT 15:48:10 ON 18 JAN 2006
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FILE 'BIOSIS' ENTERED AT 15:48:10 ON 18 JAN 2006

=> fluorescent and polypeptide and oligomerize
L3 7 FLUORESCENT AND POLYPEPTIDE AND OLIGOMERIZE

=> d ab bib

L3 ANSWER 1 OF 7 MEDLINE on STN
AB The protein components of the reaction center (RC) and core light-harvesting (LH 1) complexes of photosynthetic bacteria have evolved to specifically, but non-covalently, bind bacteriochlorophyll (Bchl). The contribution to binding of specific structural elements in the protein and Bchl may be determined for the LH 1 complex because its subunit can be studied by reconstitution under equilibrium conditions. Important to the determination and utilization of such information is the characterization of the interacting molecular species. To aid in this characterization, a **fluorescent** probe molecule has been covalently attached to each of the LH 1 **polypeptides**. The **fluorescent** probes were selected for optimal absorption and emission properties in order to facilitate their unique excitation and to enable the detection of energy transfer to Bchl. Oregon Green 488 carboxylic acid and 7-diethylaminocoumarin-3-carboxylic acid seemed to fulfill these requirements. Each of these probes were utilized to derivatize the LH1 beta-**polypeptide** of Rhodobacter sphaeroides. It was demonstrated that the beta-**polypeptides** did not interact with each other in the absence of Bchl. When Bchl was present, the probe-labeled beta-**polypeptides** interacted with Bchl to form subunit-type complexes much as those formed with the native **polypeptides**. Energy transfer from the probe to Bchl occurred with a high efficiency. The alpha-**polypeptide** from LH 1 of Rb. sphaeroides and that from Rhodospirillum rubrum were also derivatized in the same manner. Since these **polypeptides** do not **oligomerize** in the absence of a beta-**polypeptide**, reversible binding of a single Bchl to a single **polypeptide** could be measured. Dissociation constants for complex formation were estimated. The relevance of these data to earlier studies of equilibria involving subunit complexes is discussed. Also involved in the photoreceptor complex of Rb. sphaeroides and Rhodobacter capsulatus is another protein referred to as PufX. Two large segments of this protein were chemically synthesized, one reproducing the amino acid sequence of the core segment predicted for Rb. sphaeroides PufX and the other reproducing the amino acid sequence predicted for the core segment of Rb. capsulatus PufX. Each **polypeptide** was covalently labeled with a

fluorescent probe and tested for energy transfer to Bchl. Each was found to bind Bchl with an affinity similar to the affinity of the LH 1 **polypeptides** for Bchl. It is suggested that PufX binds Bchl and interacts with a Bchlcalpha-**polypeptide** component of LH 1 to truncate, or interupt, the LH 1 ring adjacent to the location of the Q(B) binding site of the RC.

AN 2005552006 IN-PROCESS
DN PubMed ID: 16228601
TI Interaction of bacteriochlorophyll with the LH1 and PufX **polypeptides** of photosynthetic bacteria: use of chemically synthesized analogs and covalently attached **fluorescent** probes.
AU Law Christopher J; Chen Jennifer; Parkes-Loach Pamela S; Loach Paul A
CS Department of Biochemistry, Molecular Biology and Cell Biology, Northwestern University, Hogan Hall, Room 2-100, 2205 Tech Dr., Evanston, IL, 60208-3500, USA,. p-loach@northwestern.edu
SO Photosynthesis research, (2003) 75 (3) 193-210.
Journal code: 100954728. ISSN: 0166-8595.
CY Netherlands
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS NONMEDLINE; IN-DATA-REVIEW; IN-PROCESS; NONINDEXED
ED Entered STN: 20051018
Last Updated on STN: 20051215

=> a ab bib 2-7
L4 0 A AB BIB 2-7

=> fluorescent and polypeptide and oligomerize
L5 7 FLUORESCENT AND POLYPEPTIDE AND OLIGOMERIZE

=> d ab bib 2-7

L5 ANSWER 2 OF 7 MEDLINE on STN
AB Chaperonins are multisubunit, cylinder-shaped molecular chaperones involved in folding newly synthesized **polypeptides**. Here we show that MKKS/BBS6, one of several proteins associated with Bardet-Biedl syndrome (BBS), is a Group II chaperonin-like protein that has evolved recently in animals from a subunit of the eukaryotic chaperonin CCT/TRiC, and diverged rapidly to acquire distinct functions. Unlike other chaperonins, cytosolic BBS6 does not **oligomerize**, and the majority of BBS6 resides within the pericentriolar material (PCM), a proteinaceous tube surrounding centrioles. During interphase, BBS6 is confined to the lateral surfaces of the PCM but during mitosis it relocalizes throughout the PCM and is found at the intercellular bridge. Its predicted substrate-binding apical domain is sufficient for centrosomal association, and several patient-derived mutations in this domain cause mislocalization of BBS6. Consistent with an important centrosomal function, silencing of the BBS6 transcript by RNA interference in different cell types leads to multinucleate and multicentrosomal cells with cytokinesis defects. The restricted tissue distribution of BBS6 further suggests that it may play important roles in ciliated epithelial tissues, which is consistent with the probable functions of BBS proteins in basal bodies (modified centrioles) and cilia. Our findings provide the first insight into the nature and cellular function of BBS6, and shed light on the potential causes of several ailments, including obesity, retinal degeneration, kidney dysfunction and congenital heart disease.
AN 2005100721 MEDLINE
DN PubMed ID: 15731008
TI MKKS/BBS6, a divergent chaperonin-like protein linked to the obesity disorder Bardet-Biedl syndrome, is a novel centrosomal component required for cytokinesis.
AU Kim Jun Chul; Ou Young Y; Badano Jose L; Esmail Muneer A; Leitch Carmen C; Fiedrich Elsa; Beales Philip L; Archibald John M; Katsanis Nicholas;

Rattner Jerome B; Leroux Michel R
CS Department of Molecular Biology and Biochemistry, Simon Fraser University, 8888 University Drive, Burnaby, BC, V5A 1S6, Canada.
NC HD042260 (NICHD)
SO Journal of cell science, (2005 Mar 1) 118 (Pt 5) 1007-20.
Journal code: 0052457. ISSN: 0021-9533.
CY England: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200507
ED Entered STN: 20050301
Last Updated on STN: 20050727
Entered Medline: 20050726

L5 ANSWER 3 OF 7 MEDLINE on STN
AB In most organisms, high affinity ammonium uptake is catalyzed by members of the ammonium transporter family (AMT/MEP/Rh). A single point mutation (G458D) in the cytosolic C terminus of the plasma membrane transporter LeAMT1;1 from tomato leads to loss of function, although mutant and wild type proteins show similar localization when expressed in yeast or plant protoplasts. Co-expression of LeAMT1;1 and mutant in Xenopus oocytes inhibited ammonium transport in a dominant negative manner, suggesting homo-oligomerization. In vivo interaction between LeAMT1;1 proteins was confirmed by the split ubiquitin yeast two-hybrid system. LeAMT1;1 is isolated from root membranes as a high molecular mass oligomer, converted to a approximately 35-kDa **polypeptide** by denaturation. To investigate interactions with the LeAMT1;2 paralog, co-localizing with LeAMT1;1 in root hairs, LeAMT1;2 was characterized as a lower affinity NH4+ uniporter. Co-expression of wild types with the respective G458D/G465D mutants inhibited ammonium transport in a dominant negative manner, supporting the formation of heteromeric complexes in oocytes. Thus, in yeast, oocytes, and plants, ammonium transporters are able to **oligomerize**, which may be relevant for regulation of ammonium uptake.
AN 2003538443 MEDLINE
DN PubMed ID: 12952951
TI Homo- and hetero-oligomerization of ammonium transporter-1 NH4 uniporters.
AU Ludewig Uwe; Wilken Stephanie; Wu Binghua; Jost Wolfgang; Obrdlik Petr; El Bakkoury Mohamed; Marini Anne-Marie; Andre Bruno; Hamacher Tanja; Boles Eckhard; von Wiren Nicolaus; Frommer Wolf B
CS Zentrum fur Molekularbiologie der Pflanzen, Pflanzenphysiologie, Universitat Tubingen, Auf der Morgenstelle 1, 72076 Tubingen, Germany.
SO Journal of biological chemistry, (2003 Nov 14) 278 (46) 45603-10.
Electronic Publication: 2003-09-02.
Journal code: 2985121R. ISSN: 0021-9258.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200312
ED Entered STN: 20031119
Last Updated on STN: 20031225
Entered Medline: 20031224

L5 ANSWER 4 OF 7 MEDLINE on STN
AB Perfringolysin O (PFO), a water-soluble monomeric cytolysin secreted by pathogenic Clostridium perfringens, **oligomerizes** and forms large pores upon encountering cholesterol-containing membranes. Whereas all pore-forming bacterial toxins examined previously have been shown to penetrate the membrane using a single amphipathic beta hairpin per **polypeptide**, cysteine-scanning mutagenesis and multiple independent fluorescence techniques here reveal that each PFO monomer contains a second domain involved in pore formation, and that each of the

two amphipathic beta hairpins completely spans the membrane. In the soluble monomer, these transmembrane segments are folded into six alpha helices. The insertion of two transmembrane hairpins per toxin monomer and the major change in secondary structure are striking and define a novel paradigm for the mechanism of membrane insertion by a cytolytic toxin.

AN 2000021616 MEDLINE
DN PubMed ID: 10555145
TI The mechanism of membrane insertion for a cholesterol-dependent cytolysin: a novel paradigm for pore-forming toxins.
AU Shatursky O; Heuck A P; Shepard L A; Rossjohn J; Parker M W; Johnson A E; Tweten R K
CS Department of Microbiology and Immunology, The University of Oklahoma Health Sciences Center, Oklahoma City 73190, USA.
NC AI37657 (NIAID)
SO Cell, (1999 Oct 29) 99 (3) 293-9.
Journal code: 0413066. ISSN: 0092-8674.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199911
ED Entered STN: 20000111
Last Updated on STN: 20000111
Entered Medline: 19991123

L5 ANSWER 5 OF 7 CAPLUS COPYRIGHT 2006 ACS on STN
AB The protein components of the reaction center (RC) and core light-harvesting (LH 1) complexes of photosynthetic bacteria have evolved to specifically, but non-covalently, bind bacteriochlorophyll (Bchl). The contribution to binding of specific structural elements in the protein and Bchl may be determined for the LH 1 complex because its subunit can be studied by reconstitution under equilibrium conditions. Important to the determination and utilization of such information is the characterization of the interacting mol. species. To aid in this characterization, a **fluorescent** probe mol. has been covalently attached to each of the LH 1 **polypeptides**. The **fluorescent** probes were selected for optimal absorption and emission properties in order to facilitate their unique excitation and to enable the detection of energy transfer to Bchl. Oregon Green 488 carboxylic acid and 7-diethylaminocoumarin-3-carboxylic acid seemed to fulfill these requirements. Each of these probes were utilized to derivatize the LH1 β- **polypeptide** of Rhodobacter sphaeroides. It was demonstrated that the β- **polypeptides** did not interact with each other in the absence of Bchl. When Bchl was present, the probe-labeled β- **polypeptides** interacted with Bchl to form subunit-type complexes much as those formed with the native **polypeptides**. Energy transfer from the probe to Bchl occurred with a high efficiency. The α- **polypeptide** from LH 1 of Rb. sphaeroides and that from Rhodospirillum rubrum were also derivatized in the same manner. Since these **polypeptides** do not **oligomerize** in the absence of a β- **polypeptide**, reversible binding of a single Bchl to a single **polypeptide** could be measured. Dissociation consts. for complex formation were estimated The relevance of these data to earlier studies of equilibrium involving subunit complexes is discussed. Also involved in the photoreceptor complex of Rb. sphaeroides and Rhodobacter capsulatus is another protein referred to as PufX. Two large segments of this protein were chemical synthesized, one reproducing the amino acid sequence of the core segment predicted for Rb. sphaeroides PufX and the other reproducing the amino acid sequence predicted for the core segment of Rb. capsulatus PufX. Each **polypeptide** was covalently labeled with a **fluorescent** probe and tested for energy transfer to Bchl. Each was found to bind Bchl

with an affinity similar to the affinity of the LH 1 **polypeptides** for Bchl. It is suggested that PufX binds Bchl and interacts with a Bchlcα- **polypeptide** component of LH 1 to truncate, or interrupt, the LH 1 ring adjacent to the location of the QB binding site of the RC.

AN 2003:410536 CAPLUS
DN 139:320173
TI Interaction of bacteriochlorophyll with the LH1 and PufX **polypeptides** of photosynthetic bacteria: use of chemically synthesized analogs and covalently attached **fluorescent** probes
AU Law, Christopher J.; Chen, Jennifer; Parkes-Loach, Pamela S.; Loach, Paul A.
CS Department of Biochemistry, Molecular Biology and Cell Biology, Northwestern University, Evanston, IL, 60208-3500, USA
SO Photosynthesis Research (2003), 75(3), 193-210
CODEN: PHRSDI; ISSN: 0166-8595
PB Kluwer Academic Publishers
DT Journal
LA English
RE.CNT 64 THERE ARE 64 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 6 OF 7 CAPLUS COPYRIGHT 2006 ACS on STN
AB Non-oligomerizing **fluorescent** proteins, which are formed by operatively linking two or more monomers of a **fluorescent** protein, or which are derived from a **fluorescent** protein having at least one mutation that reduces or eliminates the ability of the **fluorescent** protein to **oligomerize**, are provided. The non-oligomerizing **fluorescent** proteins can be derived from a naturally occurring green **fluorescent** protein, a red **fluorescent** protein, or other **fluorescent** protein, or a **fluorescent** protein related thereto. Also provided is a fusion protein, which includes a non-oligomerizing **fluorescent** protein linked to at least one **polypeptide** of interest. In addition, a polynucleotide encoding a non-oligomerizing **fluorescent** protein is provided, as is a recombinant nucleic acid mol., which includes polynucleotide encoding a non-oligomerizing **fluorescent** protein operatively linked to at least a second polynucleotide. Vectors and host cells containing such polynucleotides also are provided, as are kits containing one or more non-oligomerizing **fluorescent** proteins or encoding polynucleotides or constructs derived therefrom. Further provided are methods of making and using the proteins and polynucleotides, e.g., to determine pH in cells or biol. tissues, to determine the presence or activity of enzymes, to identify agents or conditions which regulate the activity of an expression control sequence, and to identify interactions of a first and second mol. Thus, residues 206-alanine, 221-leucine, and 223-phenylalanine were shown to be involved in oligomerization of GFP. Substitution of charged amino acids (e.g., lysine, arginine) for these residues prevented oligomerization.

AN 2002:676160 CAPLUS
DN 137:213258
TI Non-oligomerizing **fluorescent** proteins and their uses
IN Tsien, Roger Y.; Baird, Geoffrey S.; Campbell, Robert E.; Zacharias, David A.
PA The Regents of the University of California, USA
SO PCT Int. Appl., 117 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 6

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 2002068605	A2	20020906	WO 2002-US6063	20020226

WO 2002068605 A3 20030220
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG
US 2003170911 A1 20030911 US 2001-794308 20010226
US 2003032088 A1 20030213 US 2001-866538 20010524
US 6852849 B2 20050208
CA 2439400 AA 20020906 CA 2002-2439400 20020226
JP 2004530423 T2 20041007 JP 2002-568701 20020226
PRAI US 2001-794308 A 20010226
US 2001-866538 A 20010524
WO 2002-US6063 W 20020226

L5 ANSWER 7 OF 7 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
AB The protein components of the reaction center (RC) and core light-harvesting (LH 1) complexes of photosynthetic bacteria have evolved to specifically, but non-covalently, bind bacteriochlorophyll (Bchl). The contribution to binding of specific structural elements in the protein and Bchl may be determined for the LH 1 complex because its subunit can be studied by reconstitution under equilibrium conditions. Important to the determination and utilization of such information is the characterization of the interacting molecular species. To aid in this characterization, a **fluorescent** probe molecule has been covalently attached to each of the LH 1 **polypeptides**. The **fluorescent** probes were selected for optimal absorption and emission properties in order to facilitate their unique excitation and to enable the detection of energy transfer to Bchl. Oregon Green 488 carboxylic acid and 7-diethylaminocoumarin-3-carboxylic acid seemed to fulfill these requirements. Each of these probes were utilized to derivatize the LH1 beta-**polypeptide** of Rhodobacter sphaeroides. It was demonstrated that the beta-**polypeptides** did not interact with each other in the absence of Bchl. When Bchl was present, the probe-labeled beta-**polypeptides** interacted with Bchl to form subunit-type complexes much as those formed with the native **polypeptides**. Energy transfer from the probe to Bchl occurred with a high efficiency. The alpha-**polypeptide** from LH 1 of Rb. sphaeroides and that from Rhodospirillum rubrum were also derivatized in the same manner. Since these **polypeptides** do not **oligomerize** in the absence of a beta-**polypeptide**, reversible binding of a single Bchl to a single **polypeptide** could be measured. Dissociation constants for complex formation were estimated. The relevance of these data to earlier studies of equilibria involving subunit complexes is discussed. Also involved in the photoreceptor complex of Rb. sphaeroides and Rhodobacter capsulatus is another protein referred to as PufX. Two large segments of this protein were chemically synthesized, one reproducing the amino acid sequence of the core segment predicted for Rb. sphaeroides PufX and the other reproducing the amino acid sequence predicted for the core segment of Rb. capsulatus PufX. Each **polypeptide** was covalently labeled with a **fluorescent** probe and tested for energy transfer to Bchl. Each was found to bind Bchl with an affinity similar to the affinity of the LH 1 **polypeptides** for Bchl. It is suggested that PufX binds Bchl and interacts with a Bchlcntdotalpha-**polypeptide** component of LH 1 to truncate, or interrupt, the LH 1 ring adjacent to the location of the QB binding site of the RC.
AN 2003:395234 BIOSIS
DN PREV200300395234
TI Interaction of bacteriochlorophyll with the LH1 and PufX

polypeptides of photosynthetic bacteria: Use of chemically synthesized analogs and covalently attached **fluorescent** probes.

AU Law, Christopher J.; Chen, Jennifer; Parkes-Loach, Pamela S.; Loach, Paul A. [Reprint Author]

CS Department of Biochemistry, Molecular Biology and Cell Biology, Northwestern University, 2205 Tech Dr., Hogan Hall, Room 2-100, Evanston, IL, 60208-3500, USA
p-loach@northwestern.edu

SO Photosynthesis Research, (2003) Vol. 75, No. 3, pp. 193-210. print.
ISSN: 0166-8595 (ISSN print).

DT Article

LA English

ED Entered STN: 27 Aug 2003
Last Updated on STN: 27 Aug 2003

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=> fluorescent and polypeptide and domain and linked
L6          120 FLUORESCENT AND POLYPEPTIDE AND DOMAIN AND LINKED

=> dup remove
ENTER L# LIST OR (END):L6
PROCESSING COMPLETED FOR L6
L7           102 DUP REMOVE L6 (18 DUPLICATES REMOVED)
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=> (chromoprotein or flourescense) and domain and cnidarian and anthozoan
L1 0 (CHROMOPROTEIN OR FLOURESCENSE) AND DOMAIN AND CNIDARIAN AND ANTHOZOAN

=> (chromoprotein or flourescent) and domain and cnidarian
L2 0 (CHROMOPROTEIN OR FLOURESCENT) AND DOMAIN AND CNIDARIAN

=> (chromoprotein or flourescent) and cnidarian
L3 4 (CHROMOPROTEIN OR FLOURESCENT) AND CNIDARIAN

=> (chromoprotein or flourescense) and anthozoan
L4 4 (CHROMOPROTEIN OR FLOURESCENSE) AND ANTHOZOAN

=> d ti 1-4

L4 ANSWER 1 OF 4 CAPLUS COPYRIGHT 2004 ACS on STN
TI Engineering rapidly maturing variants of the Discosoma red fluorescent protein (DsRed) and their use as bioluminescent probes

L4 ANSWER 2 OF 4 CAPLUS COPYRIGHT 2004 ACS on STN
TI cDNAs encoding chromo/fluoroproteins from non-bioluminescent Cnidarian species or non-Pennatulacean (sea pen) species and their use

L4 ANSWER 3 OF 4 CAPLUS COPYRIGHT 2004 ACS on STN
TI cDNA and protein sequences of novel chromo/fluoroproteins from non-bioluminescent Cnidarian species or are obtained from non-Pennatulacean (sea pen) species and methods for using the same

L4 ANSWER 4 OF 4 CAPLUS COPYRIGHT 2004 ACS on STN
TI Kindling fluorescent proteins from Anthozoa and Heteractis crispa and their mutants and methods for their use

=> d ab bib 1-4

L4 ANSWER 1 OF 4 CAPLUS COPYRIGHT 2004 ACS on STN
AB Nucleic acid compns. encoding rapidly maturing fluorescent proteins, as well as non-aggregating versions thereof (and mutants thereof) as well as the proteins encoding the same, are provided. The proteins of interest are proteins that are fluorescent, where this feature arises from the interaction of two or more residues of the protein. The subject proteins are further characterized in that, in certain embodiments, they are mutants of wild type proteins that are obtained either from non-bioluminescent Cnidarian, e.g., **Anthozoan**, species or are obtained from **Anthozoan** non-Pennatulacean (sea pen) species. In certain embodiments, the subject proteins are mutants of wild type Discosoma sp. 'red' fluorescent protein. Also of interest are proteins that are substantially similar to, or mutants of, the above specific proteins. Also provided are fragments of the nucleic acids and the peptides encoded thereby, as well as antibodies to the subject proteins and transgenic cells and organisms. The subject protein and nucleic acid compns. find use in a variety of different applications. Finally, kits for use in such applications, e.g., that include the subject nucleic acid compns., are provided. Claimed sequences were not present at the time of publication.
AN 2003:511470 CAPLUS
DN 139:65739
TI Engineering rapidly maturing variants of the Discosoma red fluorescent protein (DsRed) and their use as bioluminescent probes
IN Bevis, Brooke; Glick, Benjamin
PA The University of Chicago, USA
SO PCT Int. Appl., 65 pp.
CODEN: PIXXD2

DT Patent
LA English
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 2003054158	A2	20030703	WO 2002-US40539	20021218
	WO 2003054158	A3	20031204		

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

PRAI US 2001-341723P P 20011219

L4 ANSWER 2 OF 4 CAPLUS COPYRIGHT 2004 ACS on STN

AB Nucleic acid compns. encoding novel chromo/fluoroproteins and mutants thereof, as well as the proteins encoded the same, are provided. The proteins of interest are proteins that are colored and/or fluorescent, where this feature arises from the interaction of two or more residues of the protein. The subject proteins are further characterized in that they are either obtained from non-bioluminescent Cnidarian, e.g., **Anthozoan**, species or are obtained from **Anthozoan** non-Pennatulacean (sea pen) species. More specifically, they include GFP of Heteractis crispa, Dendronephthya sp, Scolymia cubensis, Ricordea florida, Montastraea cavernosa, Condylactis gigantea, Agaricia fragilis, sequence homolog of Montrastraea annularis and RFP of Zoanthus sp., Ricordea florida, and Montastraea cavernosa. Also of interest are proteins that are substantially similar to, or mutants of, the above specific proteins. Also provided are fragments of the nucleic acids and the peptides encoded thereby, as well as antibodies to the subject proteins and transgenic cells and organisms. The subject protein and nucleic acid compns. find use in a variety of different applications. Finally, kits for use in such applications, e.g., that include the subject nucleic acid compns., are provided.

AN 2003:397030 CAPLUS
DN 138:397335
TI cDNAs encoding chromo/fluoroproteins from non-bioluminescent Cnidarian species or non-Pennatulacean (sea pen) species and their use
IN Labas, Yulii Aleksandrovich; Gurskaya, Nadezda Georgievna; Yanushevich, Yuriy; Fradkov, Arcady Fedorovich; Lukyanov, Konstantin; Lukyanov, Sergey; Matz, Mikhail Vladimirovich
PA Clontech Laboratories, Inc., USA
SO PCT Int. Appl., 88 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 2003042401	A2	20030522	WO 2002-US36499	20021112
	WO 2003042401	A3	20031120		

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RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

EP 1444245 A2 20040811 EP 2002-797104 20021112

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, SK

PRAI US 2001-332980P P 20011113

WO 2002-US36499 W 20021112

L4 ANSWER 3 OF 4 CAPLUS COPYRIGHT 2004 ACS on STN

AB Nucleic acid compns. encoding novel chromo/fluoroproteins and mutants thereof, as well as the proteins encoded by the same, are provided. The subject proteins of interest are proteins that are colored and/or fluorescent, where this feature arises from the interaction of two or more residues of the protein. The subject proteins are further characterized in that they are either obtained from non-bioluminescent Cnidarian, e.g., **Anthozoan**, species or are obtained from non-Pennatulacean (sea pen) species. Specific proteins of interest include proteins obtained from the following specific **Anthozoan** species: Anemonia majano (NFP-1), Clavularia sp. (NFP-2), Zoanthus sp. (NFP-3 & NFP-4), Discosoma striata (NFP-5), Discosoma sp. "red" (NFP-6), Anemonia sulcata (NFP-7), Discosoma sp "green" (NFP-8), and Discosoma sp. "magenta" (NFP-9). Also of interest are proteins that are substantially similar to, or mutants of, the above specific proteins. Also provided are fragments of the nucleic acids and the peptides encoded thereby, as well as antibodies to the subject proteins and transgenic cells and organisms. The subject protein and nucleic acid compns. find use in a variety of different applications. Finally, kits for use in such applications, e.g., that include the subject nucleic acid compns., are provided.

AN 2002:978391 CAPLUS

DN 138:50935

TI cDNA and protein sequences of novel chromo/fluoroproteins from non-bioluminescent Cnidarian species or are obtained from non-Pennatulacean (sea pen) species and methods for using the same

IN Lukyanov, Sergey A.; Fradkov, Arcady F.; Labas, Yulii A.; Matz, Mikhail V.; Terskikh, Alexey

PA Russia

SO U.S. Pat. Appl. Publ., 48 pp., Cont.-in-part of Appl. No. PCT/US00/28477. CODEN: USXXCO

DT Patent

LA English

FAN.CNT 16

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI US 2002197676	A1	20021226	US 2001-6922	20011204
WO 2000034526	A1	20000615	WO 1999-US29405	19991210

W: JP

RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE

WO 2001027150	A2	20010419	WO 2000-US28477	20001013
WO 2001027150	A3	20011206		

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WO 2002068459	A2	20020906	WO 2002-US5749	20020220
WO 2002068459	A3	20031127		

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RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

	US 2003022287	A1	20030130	US 2002-81864	20020220
	EP 1385967	A2	20040204	EP 2002-723238	20020220

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	US 2003092884	A1	20030515	US 2002-155809	20020524
PRAI	US 1999-418529	A2	19991014		
	US 1999-418917	B2	19991015		
	US 1999-418922	B2	19991015		
	US 1999-444338	B2	19991119		
	US 1999-444341	B2	19991119		
	US 1999-457556	B2	19991209		
	US 1999-457898	B2	19991209		
	US 1999-458144	B2	19991209		
	US 1999-458477	B2	19991209		
	WO 1999-US29405	W	19991210		
	US 2000-211607P	P	20000614		
	US 2000-211609P	P	20000614		
	US 2000-211626P	P	20000614		
	US 2000-211627P	P	20000614		
	US 2000-211687P	P	20000614		
	US 2000-211766P	P	20000614		
	US 2000-211880P	P	20000614		
	US 2000-211888P	P	20000614		
	US 2000-212070P	P	20000614		
	WO 2000-US28477	A2	20001013		
	US 1998-210330	A	19981211		
	US 2001-270983P	P	20010221		
	US 2001-293752P	P	20010525		
	US 2001-329176P	P	20011011		
	US 2001-976673	A	20011012		
	US 2001-6922	A	20011204		
	WO 2002-US5749	W	20020220		

L4 ANSWER 4 OF 4 CAPLUS COPYRIGHT 2004 ACS on STN

AB Kindling fluorescent protein (KFP) compns. and nucleic acids encoding the same, as well as methods for using the same, are provided. In particular, protein FP595 from Anthozoa (also called AsFP595, or FP7, or KFP04) and its two mutants (A148G, and F90L-A148G-H203Y resp.), and another Heteractis crispa **chromoprotein** FP10 (KFP08) and its four mutants (a:K28M-N165A, b:K28M-N165G, c:G20C-T39A-L126H-C148A-N165G-R176H-L181H-A190V-I203H-P208L-K211E, and d:T39A-C148S-N165S-L181H-1203H-P208R-K211E resp.) are provided. These wild-type or mutant kindling fluorescent proteins are expressed recombinantly (as his6 epitope tagged fusion proteins) and purified for further characterization. In general, they become brightly fluorescent proteins, from an initial non-fluorescent or low fluorescent state, upon exposure to a kindling stimulus, which fluorescent state may be reversible or irreversible. Specifically, their kindling wavelength of said kindling stimulus ranges from about 200 to 1500 nm, their kindling stimulus ranges from about 0.01 to about 106 W/cm2, and their kindling duration of said kindling stimulus ranges from about 1 ms to about 60 min. The subject protein/nucleic acid compns. find use in labeling protocols, e.g., in labeling proteins, organelles, cells and organisms, etc., in a variety of different types of applications. Also provided are systems and kits for use in practicing such applications. The use of a KFP to study cell migration during

embryogenesis, and to study migration of a mitochondrion are demonstrated.
AN 2002:927445 CAPLUS
DN 138:21184
TI Kindling fluorescent proteins from Anthozoa and Heteractis crispa and their mutants and methods for their use
IN Lukyanov, Sergey Anatolievich; Chudakov, Dmitry; Lukyanov, Konstantin
PA Clontech Laboratories, Inc., USA
SO PCT Int. Appl., 96 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 16

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 2002096924	A1	20021205	WO 2002-US16379	20020524
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
EP 1390379	A1	20040225	EP 2002-746443	20020524
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR				
PRAI US 2001-293752P	P	20010525		
US 2001-329176P	P	20011011		
WO 2002-US16379	W	20020524		

RE.CNT 3 THERE ARE 3 CITED REFERENCES AVAILABLE FOR THIS RECORD
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COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
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DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)	SINCE FILE ENTRY	TOTAL SESSION
CA SUBSCRIBER PRICE	-2.94	-2.94

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FILE CONTAINS CURRENT INFORMATION.
LAST RELOADED: Aug 6, 2004 (20040806/UP).

=> index bioscience
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CA SUBSCRIBER PRICE	0.00	-2.94

INDEX 'ADISCTI, ADISINSIGHT, ADISNEWS, AGRICOLA, AQUALINE, ANABSTR, ANTE, AQUASCI, BIOBUSINESS, BIOCOMMERCE, BIOENG, BIOSIS, BIOTECHABS, BIOTECHDS,

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       BIOTECHNO, CABA, CANCERLIT, CAPLUS, CEABA-VTB, CEN, CIN, CONFSCI, CROPB,
       CROPU, DISSABS, DDFB, DDFU, DGENE, ...' ENTERED AT 12:44:25 ON 11 AUG 2004

74 FILES IN THE FILE LIST IN STNINDEX

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=> (chromoprotein or flourescent)  and anthozoan
          6   FILE BIOTECHABS
          6   FILE BIOTECHDS
          4   FILE CAPLUS
  19 FILES SEARCHED...
         48   FILE DGENE
  34 FILES SEARCHED...
          2   FILE IFIPAT
  52 FILES SEARCHED...
          1   FILE TOXCENTER
         18   FILE USPATFULL
          1   FILE USPAT2
          5   FILE WPIDS
  72 FILES SEARCHED...
          5   FILE WPINDEX

  10 FILES HAVE ONE OR MORE ANSWERS,   74 FILES SEARCHED IN STNINDEX

L5   QUE (CHROMOPROTEIN OR FLOURESCENT) AND ANTHOZOAN

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FULL ESTIMATED COST                                 2.28      50.68

DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)    SINCE FILE      TOTAL
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CA SUBSCRIBER PRICE                                 0.00      -2.94

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=> (chromoprotein or flourescent)  and anthozoan
L6          55 (CHROMOPROTEIN OR FLOURESCENT) AND ANTHOZOAN

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=> d ti 1-20

L6    ANSWER 1 OF 55  BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
TI    Novel nucleic acid encoding interconverted mutant of chromo-or
      fluorescent protein which are useful as biosensors, coloring agents;
         involving vector-mediated gene transfer and expression in host cell
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for use in transgenic plant and transgenic animal construction

L6 ANSWER 2 OF 55 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
TI Novel nucleic acid encoding a rapidly maturing chromo- or fluorescent mutant of a Cnidarian chromo- or fluorescent protein or its mutant, useful for applications involving chromo- or fluorescent proteins; involving vector-mediated gene transfer and expression in Escherichia coli

L6 ANSWER 3 OF 55 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
TI New nucleic acid encoding polypeptide products having at least two linked chromo/fluorescent domains, useful for generating transgenic plants or animals or site-specific gene modifications in cell lines; involving vector-mediated gene transfer and expression in host cell for use in color, food-additive, pharmaceutical and cosmetic industry

L6 ANSWER 4 OF 55 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
TI Novel nucleic acid that is present in other than its natural environment and that encodes kindling fluorescent protein, is useful in labeling protocols, e.g. labeling proteins, organelles, cells and organisms; vector-mediated recombinant protein gene transfer and expression in host cell for use in fluorescence resonance energy transfer, luminescence resonance energy transfer, high throughput screening and cell sorting

L6 ANSWER 5 OF 55 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
TI New nucleic acids encoding **chromoproteins** or fluorescent proteins, useful as labeling tools for marking a protein, cell or organism, which may be used in biochemistry, molecular biology or medical diagnostic applications; vector-mediated recombinant protein gene transfer and expression in host cell for use as a coloring agent and pigment and food, pharmaceutical and cosmetic industry

L6 ANSWER 6 OF 55 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
TI Novel nucleic acid encoding Stichodactylidaen **chromoprotein** and its fluorescent mutant useful as coloring agent, labels in analyte detection assays, markers in recombinant DNA applications and filters in sunscreens; vector-mediated recombinant protein gene transfer and expression in host cell, antibody and transgenic animal model construction for use in food, pharmaceutical and cosmetic industries

L6 ANSWER 7 OF 55 DGENE COPYRIGHT 2004 THOMSON DERWENT on STN
TI Novel nucleic acid encoding interconverted mutant of chromo-or fluorescent protein which are useful as biosensors, coloring agents.

L6 ANSWER 8 OF 55 DGENE COPYRIGHT 2004 THOMSON DERWENT on STN
TI Novel nucleic acid encoding interconverted mutant of chromo-or fluorescent protein which are useful as biosensors, coloring agents.

L6 ANSWER 9 OF 55 DGENE COPYRIGHT 2004 THOMSON DERWENT on STN
TI Novel nucleic acid encoding interconverted mutant of chromo-or fluorescent protein which are useful as biosensors, coloring agents.

L6 ANSWER 10 OF 55 DGENE COPYRIGHT 2004 THOMSON DERWENT on STN
TI Novel nucleic acid encoding interconverted mutant of chromo-or fluorescent protein which are useful as biosensors, coloring agents.

L6 ANSWER 11 OF 55 DGENE COPYRIGHT 2004 THOMSON DERWENT on STN
TI Novel nucleic acid encoding interconverted mutant of chromo-or fluorescent protein which are useful as biosensors, coloring agents.

L6 ANSWER 12 OF 55 DGENE COPYRIGHT 2004 THOMSON DERWENT on STN

TI Novel nucleic acid encoding interconverted mutant of chromo-or fluorescent protein which are useful as biosensors, coloring agents.

L6 ANSWER 13 OF 55 DGENE COPYRIGHT 2004 THOMSON DERWENT on STN
TI Novel nucleic acid encoding interconverted mutant of chromo-or fluorescent protein which are useful as biosensors, coloring agents.

L6 ANSWER 14 OF 55 DGENE COPYRIGHT 2004 THOMSON DERWENT on STN
TI Novel nucleic acid encoding interconverted mutant of chromo-or fluorescent protein which are useful as biosensors, coloring agents.

L6 ANSWER 15 OF 55 DGENE COPYRIGHT 2004 THOMSON DERWENT on STN
TI Novel nucleic acid encoding interconverted mutant of chromo-or fluorescent protein which are useful as biosensors, coloring agents.

L6 ANSWER 16 OF 55 DGENE COPYRIGHT 2004 THOMSON DERWENT on STN
TI Novel nucleic acid encoding interconverted mutant of chromo-or fluorescent protein which are useful as biosensors, coloring agents.

L6 ANSWER 17 OF 55 DGENE COPYRIGHT 2004 THOMSON DERWENT on STN
TI Novel nucleic acid encoding interconverted mutant of chromo-or fluorescent protein which are useful as biosensors, coloring agents.

L6 ANSWER 18 OF 55 DGENE COPYRIGHT 2004 THOMSON DERWENT on STN
TI Novel nucleic acid encoding interconverted mutant of chromo-or fluorescent protein which are useful as biosensors, coloring agents.

L6 ANSWER 19 OF 55 DGENE COPYRIGHT 2004 THOMSON DERWENT on STN
TI Novel nucleic acid encoding interconverted mutant of chromo-or fluorescent protein which are useful as biosensors, coloring agents.

L6 ANSWER 20 OF 55 DGENE COPYRIGHT 2004 THOMSON DERWENT on STN
TI Novel nucleic acid encoding interconverted mutant of chromo-or fluorescent protein which are useful as biosensors, coloring agents.

=> file biotechabs biotechds toxcenter

COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	3.09	53.77

DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)	SINCE FILE ENTRY	TOTAL SESSION
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=> (chromoprotein or flourescent) and anthozoan
L7 7 (CHROMOPROTEIN OR FLOURESCENT) AND ANTHOZOAN

=> d ti 1-7

L7 ANSWER 1 OF 7 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
TI Novel nucleic acid encoding interconverted mutant of chromo-or fluorescent protein which are useful as biosensors, coloring agents;

involving vector-mediated gene transfer and expression in host cell for use in transgenic plant and transgenic animal construction

L7 ANSWER 2 OF 7 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
TI Novel nucleic acid encoding a rapidly maturing chromo- or fluorescent mutant of a Cnidarian chromo- or fluorescent protein or its mutant, useful for applications involving chromo- or fluorescent proteins; involving vector-mediated gene transfer and expression in Escherichia coli

L7 ANSWER 3 OF 7 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
TI New nucleic acid encoding polypeptide products having at least two linked chromo/fluorescent domains, useful for generating transgenic plants or animals or site-specific gene modifications in cell lines; involving vector-mediated gene transfer and expression in host cell for use in color, food-additive, pharmaceutical and cosmetic industry

L7 ANSWER 4 OF 7 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
TI Novel nucleic acid that is present in other than its natural environment and that encodes kindling fluorescent protein, is useful in labeling protocols, e.g. labeling proteins, organelles, cells and organisms; vector-mediated recombinant protein gene transfer and expression in host cell for use in fluorescence resonance energy transfer, luminescence resonance energy transfer, high throughput screening and cell sorting

L7 ANSWER 5 OF 7 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
TI New nucleic acids encoding **chromoproteins** or fluorescent proteins, useful as labeling tools for marking a protein, cell or organism, which may be used in biochemistry, molecular biology or medical diagnostic applications; vector-mediated recombinant protein gene transfer and expression in host cell for use as a coloring agent and pigment and food, pharmaceutical and cosmetic industry

L7 ANSWER 6 OF 7 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
TI Novel nucleic acid encoding Stichodactylidaen **chromoprotein** and its fluorescent mutant useful as coloring agent, labels in analyte detection assays, markers in recombinant DNA applications and filters in sunscreens; vector-mediated recombinant protein gene transfer and expression in host cell, antibody and transgenic animal model construction for use in food, pharmaceutical and cosmetic industries

L7 ANSWER 7 OF 7 TOXCENTER COPYRIGHT 2004 ACS on STN
TI Kindling fluorescent proteins from Anthozoa and Heteractis crispa and their mutants and methods for their use

=> d ab bib 1-7

L7 ANSWER 1 OF 7 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
AB DERWENT ABSTRACT:
NOVELTY - Nucleic acid encoding an interconverted mutant (I) of a chromo- or fluorescent protein, is new.
DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1) a fragment of a nucleic acid encoding (I); (2) a construct comprising a vector and a nucleic acid encoding (I); (3) an expression cassette (II) comprises, a transcriptional initiation region that is functional in an expression host, a nucleic acid encoding (I) and a transcriptional termination region functional in the expression host; (4) a cell or its progeny comprising (II), as part of an extrachromosomal element or integrated into the genome of a host cell as a result of introduction of (II) into the host cell; (5) producing a chromo and/or fluorescent

protein, comprises, growing the cell where protein is expressed and isolating the protein substantially free of other proteins; (6) a protein (III) or its fragment encoded by the nucleic acid encoding (I) and an antibody binding specifically to the (III); (7) transgenic cell or its progeny comprises a transgene which is a nucleic acid encoding (I); (8) a kit comprising a nucleic acid encoding (I); (9) preparation (M1) of nucleic acid encoding (I); and (10) a nucleic acid produced by (M1).

BIOTECHNOLOGY - Preparation: Producing a nucleic acid that encodes a protein having at least one point mutation chosen from positions 148 and 165 as compared to the parent protein produced by the nucleic acid encoding (I). The produced nucleic acid encodes a protein having point mutations at both positions 148 and 165. (I) is the fluorescent mutant of parent non-fluorescent **chromoprotein.** The nucleic acid produced encodes a protein further comprising mutations at positions 167 and 203, where (I) is a non-fluorescent **chromoprotein** of a parent fluorescent protein (claimed). Preferred Nucleic Acid: The chromo or fluorescent protein is from a non-bioluminescent Cnidarian sp and belongs to **Anthozoan** sp.. (I) includes a point mutation chosen from a mutation at positions 148 and 165. (I) is a fluorescent mutant of a **chromoprotein** and includes a point mutation at both positions 148 and 165. (I) is a non-fluorescent **chromoprotein** of a fluorescent protein and includes a point mutation at positions 167 and 203.

USE - Nucleic acid encoding (I) is useful in any application that employs a chromo- or fluorescent protein. (III) is useful in any application that employs a chromo- or fluorescent protein (claimed). Nucleic acid encoding (I) is useful in the generation of transgenic, non-human plants or animals or site specific gene modification in cell lines. **Chromoprotein** encoded by the nucleic acid is useful as coloring agents which are capable of imparting color or pigment to a particular composition of matter e.g. food compositions, pharmaceuticals, cosmetics, living organisms, etc. The **chromoprotein** is also useful as labels in biological analyte detection assays and as selectable markers in recombinant DNA applications (e.g. the production of transgenic cells and organisms) and is also useful as sunscreens, selective filters, etc. The fluorescent protein encoded by the nucleic acid, is useful in fluorescence resonance energy transfer (FRET) applications and also useful as biosensors in prokaryotic and eukaryotic cells e.g. as Ca2+ ion indicator and as marker of whole cells to detect changes in multicellular reorganization and migration. The fluorescent proteins are also useful as second messenger detector, e.g. by fusing the subject proteins to specific domains (Protein Kinase C gamma calcium binding domain) and as in vivo marker in animals (e.g. transgenic animals). The fluorescent proteins are also useful in fluorescence activated cell sorting application, in protease cleavage assays and in assays to determine the phospholipid composition in biological membranes. The fluorescent protein is a fluorescent timer, where the switch of one fluorescent color to another (e.g. green to red) concomitant with the aging of fluorescent protein, is used to determine the activation or deactivation of gene expression.

EXAMPLE - A purple **chromoprotein**, asCP from Anemonia sulcata and a red fluorescent protein DsRed from Discosoma Sp. were selected as representatives of **chromoprotein** (CP) and fluoroprotein (FP) respectively. Site directed and random mutagenesis were performed to transform CP into FP and vice versa. Site directed mutagenesis was performed by PCR with primers containing target substitution using the overlap extension method. All mutants were cloned into pQE30 vector so that recombinant proteins contained 6-histidine tag at their N-termini. Escherichia coli XL1 Blue cells were transformed with the plasmids according to standard protocols and spread onto 3-4 Petri dishes with LB agar media supplemented with ampicillin for selection. After overnight growth at 37degreesC the plates were stored for 2-5 days at room temperature or 4degreesC to allow proteins to mature completely. The plates were washed with phosphate buffered saline (PBS). Cells were

disrupted by sonication and soluble recombinant proteins were purified on the TALON metal affinity resin. Absorption spectra of the proteins were recorded on a Beckman DU520 UV/VIS spectrophotometer. The amino acid substitution in asCP mutant in the positions 148 and 165, increased quantum yield of red fluorescence. Serine and valine were substituted at positions 148 and 165 respectively. In fluorescent DsRed, substitutions at position 148, 165, 167 and 203 significantly decreased fluorescence intensity, and the spectral characteristics of these mutants became more close to those of CPs. Non-fluorescent (NF) mutant DsRed-NF carried four amino acid substitutions, specifically. Ser148Cys, Ile165Asn, Lys167Met and Ser203Ala. DsRed-NF possessed a high extinction coefficient and an extremely low quantum yield (less than 0.001). These special characteristics converted DsRed-NF into a true **chromoprotein.**
(56 pages)

AN 2003-22525 BIOTECHDS

TI Novel nucleic acid encoding interconverted mutant of chromo-or fluorescent protein which are useful as biosensors, coloring agents; involving vector-mediated gene transfer and expression in host cell for use in transgenic plant and transgenic animal construction

AU BULINA M E; CHUDAKOV D; LUKYANOV K A

PA CLONTECH LAB INC

PI WO 2003057833 17 Jul 2003

AI WO 2002-US41418 23 Dec 2002

PRAI US 2001-343128 26 Dec 2001; US 2001-343128 26 Dec 2001

DT Patent

LA English

OS WPI: 2003-607998 [57]

L7 ANSWER 2 OF 7 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN

AB DERWENT ABSTRACT:

NOVELTY - A nucleic acid (I) that encodes a rapidly maturing chromo or fluorescent mutant of a Cnidarian chromo- or fluorescent protein or its mutant, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1) a fragment (II) of (I); (2) a construct (III) comprising a vector and (I); (3) an expression cassette (IV) comprising, a transcriptional initiation region functional in an expression host, (I), or (II), and a transcriptional termination region functional in the expression host; (4) a cell (V), or its progeny, comprising (IV) as part of an extrachromosomal element or integrated into the genome of a host cell as a result of introduction of the expression cassette into the host cell; (5) a protein (VI) or its fragment encoded by (I); (6) an antibody (VII) binding specifically to (VI); (7) a transgenic cell or its progeny, or a transgenic organism comprising a transgene that is (I) or (II); and (8) a kit comprising (I) or (II).

WIDER DISCLOSURE - (1) homologs of (I); (2) nucleic acids that encode proteins as (I), but differ in sequence of (I) due to degeneracy of genetic code; (3) nucleic acid that hybridize to (I); (4) nucleic acid that encode fusion proteins comprising (VI) and a heterologous protein; (5) homologs or fragments of (VI); and (6) polypeptides that differ from (VI).

BIOTECHNOLOGY - Preferred Nucleic Acid: (I) encodes a chromo- or fluorescent mutant of a non-bioluminescent Cnidarian species (e.g. **Anthozoan** species, preferably Discosoma sp.) chromo- or fluorescent protein. More preferably, (I) encodes a mutant of wild-type Discosoma sp. red fluorescent protein (DsRed), where the nucleic acid encodes a product having a point mutation at at least one of position 2, 5, 6, 21, 41, 42, 44 and 117 relative to wild-type DsRed, preferably a product having a point mutation at at least one of position 145 and 217. The nucleic acid encoding the product has a sequence of residues that is substantially the same or identical to a nucleotide sequence of at least 10 residues in length of a fully defined wild-type DsRed nucleic acid sequence (S1) as given in the specification.

USE - (I) is useful in applications involving nucleic acid encoding

a chromo- or fluorescent protein. (V) is useful for producing a chromo and/or fluorescent protein which involves growing the cell, whereby the protein is expressed, and isolating the protein substantially free of other proteins. (VI) is useful in applications involving chromo- or fluorescent protein (claimed). (I) is useful as PCR primers, hybridization probes, etc. The expression cassettes are useful for synthesizing (VI). The **chromoproteins** are useful as coloring agents which are capable of imparting color or pigment to a particular composition of matter e.g. food compositions, pharmaceuticals, cosmetics, living organisms, e.g., animals and plants. The **chromoproteins** may also find use as labels in analyte detection assays, e.g. assays for biological analytes of interest and as selectable markers in recombinant DNA applications, e.g. the production of transgenic cells and organisms. The fluorescent proteins find use in a variety of different applications, e.g. in fluorescence resonance energy transfer (FRET) applications, as biosensors in prokaryotic and eukaryotic cells, in applications involving the automated screening of arrays of cells expressing fluorescent reporting groups by using microscopic imaging and electronic analysis, as second messenger detectors, and in fluorescence activated cell sorting applications and as in vivo marker in animals. The fluorescent proteins also find use in protease cleavage assays. The proteins can also be used is assays to determine the phospholipid composition in biological membranes and as a fluorescent timer.

EXAMPLE - Wild-type Discosoma sp. red fluorescent protein (DsRed), an orange-red fluorescence with an emission maximum at 583 nm, had several problems for use as a fluorescent reporter, e.g., slow maturation. Therefore, to identify rapidly maturing DsRed variants, an earlier method for visualizing green fluorescent protein (GFP) fluorescence in microbial colonies was modified. Hexahistidine-tagged DsRed was produced at high levels in Escherichia coli. The fluorescence of the bacterial colonies was excited by placing a 520 +/- 20 nm bandpass filter over the lens of a slide projector, and the emission was detected. A library of mutant expression plasmids was generated using error-prone PCR to amplify the DsRed1 template. This library was transformed into E. coli, and over 100000 transformant colonies were examined. Colonies producing the wild-type DsRed1 protein required two days to develop significant fluorescence, but three mutant colonies showed strong fluorescence after one day of growth. Sequencing revealed that the three mutant plasmids were distinct, but that all of them contained an N42H codon change. Therefore a variant was generated that had only the N42H substitution. The N42H variant was purified in parallel with DsRed1, and the two proteins were analyzed by spectrofluorometry. The spectra of purified DsRed1 changed over a period of days as the protein matured. By contrast, the spectra of the purified N42H variant remained stable over time consistent with rapid maturation. In addition to accelerating maturation, the N42H substitution altered the spectral properties of the mature protein. Mature DsRed1 was assumed to be an equilibrium mixture of red fluorescent molecules and some green fluorescent molecules that were spectrally similar to GFP. The GFP-like species had a blue excitation peak at approximately 480 nm and a green emission peak at approximately 500 nm, but DsRed was tetramer, so excitation of the green molecules often resulted in the fluorescence resonance energy transfer (FRET) with neighboring red molecules to produce and emission. The FRET effect, together with the relatively low percentage of green molecules in mature DsRed1, yielded a very small peak of green emission relative to the red emission. In the N42H variant, the peaks of blue excitation and green emission were dramatically enhanced, indicating that the equilibrium had shifted so that a target percentage of the mature molecules contained the green chromophore. Because the N42H substitution considerably increased the size of the side chain, a more conservative N42Q substitution was also tried. This mutation required two base changes and probably would not have been present in the original mutant collection. The N42Q variant retained the rapid maturation property of the N42H variant, but showed much less blue excitation and green emission. The N42Q variant was

therefore chosen as the starting point for further study. Additional mutagenesis yielded DsRed variants that showed even faster maturation and lower green emission than the original N42Q variant. After six rounds of mutagenesis, three optimized variants were selected and termed DsRed T1, DsRed T3 and DsRed T4. The spectral properties of DsRed T4 were virtually identical to those of DsRed T1 and very similar to those of the wild-type DsRed1. Compared with DsRed T1 and DsRed T4, DsRed T3 was somewhat brighter but has a significantly higher peak of blue excitation and a marginally higher peak of green emission. The optimized DsRed variants were examined both in vivo and in vitro. As judged by colony fluorescence, colony size, and plasmid stability, these variants were less toxic to E. coli than DsRed1, and they developed fluorescence more efficiently at growth temperatures of 37 degreesC and higher. Like wild-type DsRed, the optimized variants appeared to be tetrameric, they exhibited FRET between the green and red molecules. The end result was pair of optimized variants termed DsRed T3 and DsRed T4. DsRed T4, DsRed T3 matured rapidly, and the purified protein was nearly as bright as mature wild-type DsRed. Making this variant well suited to single-color imaging of red fluorescence. DsRed T3 had a higher peak of blue excitation and a slightly higher peak of green emission than wild-type DsRed. DsRed T4 had fluorescence spectra very similar to those of wild-type DsRed and yielded negligible contamination of the GFP signal. Although purified DsRed T4 was only about half as bright as DsRed T3, this effect was partially offset in vivo because DsRed T4 matures nearly twice as fast as DsRed T3. Thus, DsRed T4 was probably the best variant for most applications.(65 pages)

AN 2003-20948 BIOTECHDS

TI Novel nucleic acid encoding a rapidly maturing chromo- or fluorescent mutant of a Cnidarian chromo- or fluorescent protein or its mutant, useful for applications involving chromo- or fluorescent proteins; involving vector-mediated gene transfer and expression in Escherichia coli

AU BEVIS B; GLICK B

PA UNIV CHICAGO

PI WO 2003054158 3 Jul 2003

AI WO 2002-US40539 18 Dec 2002

PRAI US 2001-341723 19 Dec 2001; US 2001-341723 19 Dec 2001

DT Patent

LA English

OS WPI: 2003-569236 [53]

L7 ANSWER 3 OF 7 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN

AB DERWENT ABSTRACT:

NOVELTY - A nucleic acid encoding a polypeptide product comprising a first and a second chromo/fluorescent domain, optionally joined by a linking domain, is new. The first and second chromo/fluorescent domains associate with each other under intracellular conditions so that the encoded polypeptide assumes a tertiary structure.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for the following: (1) a construct comprising a vector and the above nucleic acid; (2) an expression cassette comprising transcriptional initiation and termination regions functional in an expression host, and the nucleic acid cited above; (3) a cell, or its progeny, comprising an expression cassette cited above as part of an extrachromosomal element or integrated into the genome of a host cell as a result of introduction of the cassette into the host cell; (4) producing the above polypeptide, comprising growing the cell cited above to express the polypeptide product; (5) a protein, or its fragment, encoded by the nucleic acid cited above; (6) an antibody binding specifically to the protein cited above; (7) a transgenic organism or transgenic cell or cell progeny, comprising a transgene that is the nucleic acid cited above; (8) an application that employs a chromo- or fluorescent protein or a nucleic acid encoding the chromo- or fluorescent protein, the improvement comprising employing the above protein or nucleic acid; and (9) a kit

comprising the nucleic acid cited above.

BIOTECHNOLOGY - Preferred Nucleic Acid: The first and second chromo/fluorescent domains are oligomeric producing domains. The chromo/fluorescent domains are chromo- or fluorescent proteins from a Cnidarian species or mutants of chromo- or fluorescent proteins from a Cnidarian species. The Cnidarian species is a non-bioluminiscent Cnidarian species, particularly an **Anthozoan** species. The nucleic acid encodes a fusion protein of the first and second chromo/fluorescent domains fused to a non-chromo/fluorescent protein domain.

USE - The nucleic acid and the protein are useful in producing labeled fusion proteins that have a precise and predictable signal to fusion partner ratio. The nucleic acid may also be used in generating transgenic, non-human plants or animals or site-specific gene modifications in cell lines. The **chromoproteins** may be used as coloring agents, as a food composition, in pharmaceuticals or cosmetics, as labels in analyte detection assays or as selectable markers in recombinant DNA applications. (34 pages)

AN 2003-15503 BIOTECHDS

TI New nucleic acid encoding polypeptide products having at least two linked chromo/fluorescent domains, useful for generating transgenic plants or animals or site-specific gene modifications in cell lines; involving vector-mediated gene transfer and expression in host cell for use in color, food-additive, pharmaceutical and cosmetic industry

AU LUKYANOV S A

PA CLONTECH LAB INC

PI WO 2003031590 17 Apr 2003

AI WO 2002-US32560 10 Oct 2002

PRAI US 2002-383336 22 May 2002; US 2001-976673 12 Oct 2001

DT Patent

LA English

OS WPI: 2003-381709 [36]

L7 ANSWER 4 OF 7 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN

AB DERWENT ABSTRACT:

NOVELTY - A nucleic acid (I) present in other than its natural environment, where (I) encodes a kindling fluorescent protein that goes from a first substantially non-fluorescent or non-fluorescent state to a second fluorescent state upon exposure to a kindling stimulus, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) a fragment (II) of (I); (2) a construct (III) comprising a vector and (I); (3) an expression cassette (IV) comprising a transcriptional initiation region functional in an expression host, (I), and a transcriptional termination region functional in the expression host; (4) a cell (V) or its progeny comprising (IV) as a part of an extrachromosomal element or integrated into the genome of a host cell; (5) a protein (VI) or its fragment encoded by (I); (6) an antibody (VII) binding specifically to (VI); (7) a transgenic cell (VIII) or its progeny comprising a transgene that comprises (I); (8) a transgenic organism (IX) comprising a transgene that comprises (I); (9) production of (VI); (10) producing (M) a fluorescent protein by subjecting (I) to a kindling stimulus to produce a kindled kindling fluorescent protein which is fluorescent; (11) a system (X) for producing a kindled fluorescent protein from (VI), comprises (I) or (VI), and a source of kindling stimulus; and (12) a kit (XI) comprising (I) and instructions for producing a fluorescent protein from (I).

WIDER DISCLOSURE - Also disclosed are: (1) a composition comprising (I), (V), (VI), (VII) or recombinant vectors; (2) homologs of (I) or (VI); (3) nucleic acids that encode the proteins encoded by (I), but differ in sequence from (I) due to the degeneracy of the genetic code; (4) nucleic acids that hybridize to (I) under stringent conditions; (5) nucleic acids encoding variants e.g., mutants of (I); and (6) nucleic acids that encode fusion proteins of (I) or its fragments.

BIOTECHNOLOGY - Preparation: (VI) is produced by growing (V), where

(VI) is expressed, and isolating (VI) that is substantially free of other proteins (claimed). Preferred Nucleic Acid: In (I), the kindling stimulus is light of a kindling wavelength, intensity and duration effective to kindle the kindling fluorescent protein. The kindling wavelength of the kindling stimulus ranges from about 200-1500 nm, the kindling intensity of the kindling stimulus ranges from 0.01-106 W/cm2, and the kindling duration of the kindling stimulus ranges from 1 millisecond to about 60 minutes. The kindling fluorescent protein does not have a sequence that is identical to a sequence comprising 232 or 215 amino acids fully defined in the specification. The kindling fluorescent protein is a mutant of a wild type kindling fluorescent protein. The kindling fluorescent protein is a wild type protein or its mutant from a non-bioluminescent Cnidarian species, preferably an **Anthozoan** species. The second state is transient. The second fluorescent state is permanent. (I) is an isolated nucleic acid. Preferred Method: In (M), the kindling fluorescent protein is present inside an organism or cell.

USE - (VI) is useful for detecting an entity such as a protein, organelle or cell in a composition such as a cell or a multicellular composition (preferably a multicellular organism), by providing the entity as an entity labeled with (VI), kindling the kindling fluorescent protein label with a kindling stimulus to produce a kindled kindling fluorescent protein label, and exciting the kindled kindling fluorescent protein label with light and detecting any fluorescence from it to detect the entity. The method monitors the movement of the entity (claimed). (I) or (VI) is useful in labeling protocols, e.g., labeling proteins, organelles, cells and organisms, as biological labels or markers, in protein labeling or tagging applications. (II) is useful as primers for polymerase chain reaction, as hybridization screening probes and for the production of (VI). (VI) is useful as detectable labels, as labels in analyte detection assays, in fluorescence resonance energy transfer (FRET) applications, in bioluminescence resonance energy transfer (BRET) applications, as biosensors in prokaryotic and eukaryotic cells, in applications involving the automated screening of arrays of cells expressing fluorescent reporting groups, in high through-put screening assays, as second messenger detectors, and in fluorescent activated cell sorting assays.

EXAMPLE - Generation of and initial characterization of kindling fluorescent proteins was as follows. Routine target i.e. site specific, and random mutagenesis of wild type asFP595 and Heteractis crispa **chromoproteins** was carried out. The asFP595 (asCP) had a sequence comprising 232 amino acids fully defined in the specification and was encoded by a nucleotide coding sequence comprising 767 base pairs fully defined in the specification. The H.crispa **chromoprotein** had a sequence comprising 215 amino acids fully defined in the specification was encoded by a nucleotide coding sequence comprising 760 base pairs fully defined in the specification. Mutagenesis was performed by the overlap extension method. Briefly, two overlapping fragments of each FP coding region were amplified. Polymerase chain reaction (PCR) was carried out using Advantage 2 polymerase mix in 1xmanufacturer's buffer supplemented with 100 muM of each dNTP, 0.2 muM of each primer, and 1 ng of plasmid DNA in 25 mul (final volume). To remove plasmids encoding wild type proteins, the 5' and 3'-fragments were excised from 2% low-melting agarose gel in 1xTAE buffer. To drain the DNA solution, the gel pieces were subjected to 3 freeze-thaw cycles. For each particular mutant, appropriate 5'- and 3'-fragments were combined to obtain full-length cDNA as follows. Equal volumes of 5'-fragment solution, 3'-fragment solution and 3xPCR mixture containing Advantage 2 polymerase mix, buffer and dNTPs were mixed together and subjected to 2-3 cycles of 95degreesC for 20 s, 65degreesC for 30 min, 72degreesC for 30 s. Then, the reaction was diluted 10 fold and 1 mul of the diluted sample was used as a template for PCR with forward and reverse cloning primers. As a result, ready-for-cloning fragments containing full-length coding regions with target substitution(s) were generated. Mutant PC products were digested with endonucleases, for which the cloning primers contained sites, and

then cloned into pQE30, digested with endonucleases generating complementary overhangs. Each of the recombinant proteins generated by both cloning-expression systems contained a 6xHis tag on the N- or C-terminus. Selected Escherichia coli clones were grown at 37degreesC in 50 ml to an optical density of (OD) 0.6. At that point, the expression of recombinant FP was induced with 0.2 mM isopropyl-beta-D-thiogalactoside (IPTG). The cultures were then incubated overnight. The following day, cells were harvested by centrifugation, resuspended in buffer and disrupted by sonication. Fluorescent proteins were purified from the soluble fraction. Proteins were at least 95% pure according to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). (96 pages)

AN 2003-09318 BIOTECHDS

TI Novel nucleic acid that is present in other than its natural environment, and that encodes kindling fluorescent protein, is useful in labeling protocols, e.g. labeling proteins, organelles, cells and organisms; vector-mediated recombinant protein gene transfer and expression in host cell for use in fluorescence resonance energy transfer, luminescence resonance energy transfer, high throughput screening and cell sorting

AU LUKYANOV S A; CHUDAKOV D; LUKYANOV K

PA CLONTECH LAB INC

PI WO 2002096924 5 Dec 2002

AI WO 2002-US16379 24 May 2002

PRAI US 2001-329176 11 Oct 2001; US 2001-293752 25 May 2001

DT Patent

LA English

OS WPI: 2003-156788 [15]

L7 ANSWER 5 OF 7 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN

AB DERWENT ABSTRACT:

NOVELTY - A new nucleic acid comprises: (a) nucleic acid encoding a condylactin **chromoprotein** or its fluorescent mutant; (b) nucleic acid encoding a fluorescent protein having an emission maximum ranging from about 480-680 nm; or (c) nucleic acid having a sequence that is substantially the same as or identical to a nucleotide sequence of at least 10 residues in length of 835 or 681 bp fully defined in the specification.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) fragment of the nucleic acid cited above; (2) isolated nucleic acid or its mimetic that hybridizes under stringent conditions to the nucleic acid cited above, or it complementary sequence; (3) construct comprising a vector and the nucleic acid cited above, its fragment or a nucleic acid or its complement that hybridizes under stringent conditions to the above nucleic acids; (4) expression cassette comprising: (a) transcriptional initiation region functional in an expression host; (b) nucleic acid selected from the group of nucleic acids cited above; and (c) transcriptional termination region functional in the expression host; (5) cell or its progeny comprising the expression cassette as part of an extra-chromosomal element or integrated into the genome of a host cell as a result of introduction of the expression cassette into the host cell; (6) method of producing an **anthozoan** chromo and/or fluorescent protein comprising growing a cell where the protein is expressed, and isolating the protein substantially free of other proteins; (7) protein or its fragment encoded by the nucleic acid above; (8) antibody binding specifically to the protein of (7); (9) transgenic cell or its progeny comprising a transgene selected from the nucleic acids above; (10) transgenic organism comprising a transgene selected from the nucleic acids above; and (11) kit comprising the nucleic acid and instructions for using the nucleic acid.

BIOTECHNOLOGY - Preferred Nucleic Acid: The nucleic acid is preferably isolated. The nucleic acid has a sequence similarity of at least 60% with a sequence of at least 10 residues in length of 835 or 681 bp fully defined in the specification. Preparation: The nucleic acid is prepared by standard recombinant techniques.

USE - The nucleic acids encoding **chromoproteins** or fluorescent proteins are useful as labeling tools for marking a protein, cell or organism, which may be used in biochemistry, molecular biology or medical diagnostic applications. The **chromoproteins** may be used as coloring agents capable of imparting color or pigment to a particular composition of matter, e.g. food compositions, pharmaceuticals or cosmetics. They may also be used as labels in analyte detection assays, or as selectable markers in recombinant DNA applications. The fluorescent proteins may be used in fluorescence resonance energy transfer (FRET) applications, e.g. detection of protein-protein interactions. They are also used as biosensors in prokaryotic and eukaryotic cells, e.g. as pH indicator or phosphorylation indicator. The proteins are useful as in vivo marker in animals, in assays to determine the phospholipid composition in biological membranes, or in protease cleavage assays.

EXAMPLE - The nucleic acid of 681 base pairs, fully defined in the specification, encoding a **chromoprotein** was aligned with that of Green Fluorescent Protein (GFP). Residue 148 was identified as being occupied by a Cys residue instead of a Ser residue, where Ser 148 is present in fluorescent Anthozoa derived proteins. Site-directed mutagenesis was employed to generate point mutations of the **chromoprotein** containing Ser at position 148. Mutagenesis was performed by the overlap extension method. Overlapping fragments of each FP coding region were amplified using the following primers: forward 5' ACATGGATCCGCTGGTTTGTTGAAAGA 3' ; and reverse 5' ATGTCAGTGCTTGGTTCCCAT 3'; were used for forward cloning and reverse mutagenesis of the 5'-end fragment amplification, and forward 5' ATGGGAACCAAGCACTGAGAT 3' and reverse cloning 5' TGACAAGCTTCTGGTGTCACTGGGAACAATCA 3' primers were used for 3' end fragment amplification. Polymerase Chain Reaction (PCR) was carried out using AdvantageTM2 Polymerase Mix in 1x buffer supplemented with 100microM of each dNTP, 0.2microM of each primer and 1ng of plasmid DNA in 25microl (final volume). The cycling parameters were 95degreesC for 10 seconds, 65 degreesC for 30 seconds, and 72degreesC for 30 seconds, for 20 cycles. To remove plasmids encoding wilt-type proteins, the 5' and 3' fragments were excised from 2% low-melting agarose gel in 1x TAE buffer. To drain the DNA solution, the gel pieces were subjected to 3 freeze-thaw cycles. The 5' and 3' fragments were combined to obtain full-length cDNA by PCR using the same cycling parameters. The reaction was then diluted 10 fold and 1microl of the diluted sample was used as a template for PCR with forward and reverse cloning primers as described for 5' and 3' fragments amplification. As a result, a cloning fragment containing full-length coding regions with the target substitution was generated.(56 pages)

AN 2003-02756 BIOTECHDS

TI New nucleic acids encoding **chromoproteins** or fluorescent proteins, useful as labeling tools for marking a protein, cell or organism, which may be used in biochemistry, molecular biology or medical diagnostic applications;
vector-mediated recombinant protein gene transfer and expression in host cell for use as a coloring agent and pigment and food, pharmaceutical and cosmetic industry

AU LUKYANOV S A; LUKYANOV K A; FRADKOV A F

PA CLONTECH LAB INC

PI WO 2002059309 1 Aug 2002

AI WO 2001-US47995 11 Dec 2001

PRAI US 2000-255533 13 Dec 2000; US 2000-255533 13 Dec 2000

DT Patent

LA English

OS WPI: 2002-666902 [71]

L7 ANSWER 6 OF 7 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN

AB DERWENT ABSTRACT:
NOVELTY - A nucleic acid (I) present in other than its natural environment and encoding an Stichodactylidaen **chromoprotein** or its fluorescent mutant, where the fluorescent protein has an emission

maximum ranging from 580-660 nm, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) a nucleic acid (II) having a sequence of residues that is substantially the same as or identical to a nucleotide sequence of at least 10 residues in length of a sequence (S) of 910, 908, 684, 681 or 687 base pairs given in the specification; (2) a fragment (III) of (I) or (II); (3) an isolated nucleic acid or its mimetic that hybridizes under stringent conditions to (I), (II) or their complementary sequences; (4) a construct comprising a vector and (I), (II), (III), or a nucleic acid or its complement that hybridizes under stringent conditions to the above nucleic acids; (5) an expression cassette (IV) comprising (I), (II), (III), or the above construct, and transcriptional initiation and termination region functional in an expression host; (6) a cell (V) or its progeny comprising (IV) as part of an extrachromosomal element or integrated into the genome of a host cell as a result of introduction of the expression cassette into the host cell; (7) a protein (VI) or its fragment encoded by (I), (II) or (III); (8) an antibody (VII) binding specifically to (VI); (9) a transgenic cell or its progeny comprising (I), (II) or (III); (10) a transgenic organism comprising (I), (II) or (III); and (11) a kit comprising (I), (II) or (III) and instructions for using the nucleic acid.

WIDER DISCLOSURE - Also disclosed are: (1) homologs of (I); (2) nucleic acids that encode proteins encoded by (I), but differ in sequence due to degeneracy of the genetic code; and (3) nucleic acids that encode fusion proteins comprising (VI) fused to a second protein.

BIOTECHNOLOGY - Preparation: (VI) is produced recombinantly. Preferred Nucleic Acid: (I) is isolated. (II) as a sequence similarity of 60% with sequence of 10 residues in (S).

USE - (I), (II) or (III), and (VI) are useful in applications employing a chromo or fluorescent nucleic acid or protein. (V) is useful for producing an **Anthozoan** chromo and/or fluorescent protein (claimed). (III) is useful as primers for polymerase chain reaction (PCR) and hybridization screening probes. (I) is useful to identify expression of the gene in a biological specimen, and to generate transgenic, non-human plants or animals or site specific gene modifications in cell lines. The **chromoproteins**, and their fluorescent mutants are useful as coloring agents capable of imparting color or pigment to a particular composition of matter. The **chromoproteins** can be incorporated into a variety of different compositions including food compositions, pharmaceuticals, cosmetics, living organisms, e.g. animals and plants, and as labels in analyte detection assays, e.g. assays for biological analytes of interest. The **chromoproteins** may be incorporated into adducts with analyte specific antibodies or their binding fragments and subsequently employed in immunoassays for analytes of interest in a complex sample. They are also useful as selectable markers in recombinant DNA applications, e.g. the production of transgenic cells and organisms, in sunscreens, as selective filters, and in fluorescence resonance energy transfer (FRET) applications, where the proteins serve as donor and/or acceptors in combination with a second fluorescent protein or dye, e.g. a fluorescent protein. The proteins also find use as biosensors in prokaryotic and eukaryotic cells, e.g. as Ca2+ ion indicator, as pH indicator, as phosphorylation indicator, as an indicator of other ions, e.g. magnesium, sodium, potassium, chloride and halides and in applications involving the automated screening of arrays of cells expressing fluorescent reporting groups by using microscopic imaging and electronic analysis. Screening can be used for drug discovery and in the field of functional genomics e.g. where the subject proteins are used as markers of whole cells to detect changes in multicellular reorganization and migration, e.g. formation of multicellular tubules by endothelial cells, migration of cells, wound healing and neurite outgrowth. The fluorescent protein also finds use in high throughput screening assays, in fluorescence activated cell sorting applications, as a label to mark a population of cells, as in vivo marker in animals, in protease cleavage assays, in assays to determine the phospholipid

composition in biological membranes and as a fluorescent timer, in which the switch of one fluorescent color to another (e.g. green to red) concomitant with the aging of the fluorescent protein is used to determine the activation/deactivation of gene expression, e.g. developmental gene expression, cell cycle dependent gene expression or circadian rhythm specific gene expression. (VII) is useful for differentiating the fluorescent protein from other fluorescent proteins.

EXAMPLE - A mutant **chromoprotein** C148S was generated. Upon alignment of the **chromoprotein** of 227 amino acids fully defined in the specification with green fluorescent protein (GFP), residue 148 (numbering based on GFP) was identified as being occupied by a Cys residue instead of a Ser residue, where Ser 148 was present in all of the fluorescent Anthozoa derived proteins. Site directed mutagenesis was employed to generate point mutants of the **chromoprotein** containing Ser at position 148. Mutagenesis was performed by the overlap extension method. Two overlapping fragments of each FP coding region were amplified. Forward cloning (5'-acatggatccgctggtttgttgaaaga) and reverse mutagenesis (5'-acctcagtgcttggctcccat) primers were used for 5'-end fragment amplification, and forward mutagenesis (5'-atgggagccaagcactgaggt) and reverse cloning (5'-tgacaagcttctggtgtcactgggaacaatca) primers were used for 3'-end fragment amplification. Polymerase chain reaction (PCR) was carried out using 100 microM of each dNTP, 0.2 microM of each primer and 1 ng of plasmid DNA. To remove plasmids encoding wild type proteins, the 5'- and 3'-fragments were excised. Then 5'-and 3'-fragments were combined to obtain full-length cDNA. The reaction was diluted 10 fold and 1 microl of the diluted sample was used as a template for PCR with forward and reverse cloning primers. Ready-for-cloning fragment containing full-length coding regions with target substitution was generated. This single substitution dramatically increased the quantum yield of red fluorescence as compared to the wild type protein. By random mutagenesis of the primary fluorescent mutant (with Ser 148), a brighter mutant, i.e., 44-9 (hcFRFP) (HcRed), was generated. (81 pages)

AN 2002-16017 BIOTECHDS

TI Novel nucleic acid encoding Stichodactylidaen **chromoprotein** and its fluorescent mutant useful as coloring agent, labels in analyte detection assays, markers in recombinant DNA applications and filters in sunscreens;
vector-mediated recombinant protein gene transfer and expression in host cell, antibody and transgenic animal model construction for use in food, pharmaceutical and cosmetic industries

AU LUKYANOV S A; FRADKOV A F; LUKYANOV K A; GURSKAYA N G

PA CLONTECH LAB INC

PI WO 2002030965 18 Apr 2002

AI WO 2000-US32080 12 Oct 2000

PRAI US 2001-306131 16 Jul 2001

DT Patent

LA English

OS WPI: 2002-444170 [47]

L7 ANSWER 7 OF 7 TOXCENTER COPYRIGHT 2004 ACS on STN

AB Kindling fluorescent protein (KFP) compns. and nucleic acids encoding the same, as well as methods for using the same, are provided. In particular, protein FP595 from Anthozoa (also called AsFP595, or FP7, or KFP04) and its two mutants (A148G, and F90L-A148G-H203Y resp.), and another Heteractis crispa **chromoprotein** FP10 (KFP08) and its four mutants (a:K28M-N165A, b:K28M-N165G, c:G20C-T39A-L126H-C148A-N165G-R176H-L181H-A190V-I203H-P208L-K211E, and d:T39A-C148S-N165S-L181H-1203H-P208R-K211E resp.) are provided. These wild-type or mutant kindling fluorescent proteins are expressed recombinantly (as his6 epitope tagged fusion proteins) and purified for further characterization. In general, they become brightly fluorescent proteins, from an initial non-fluorescent or low fluorescent state, upon exposure to a kindling stimulus, which fluorescent state may be reversible or irreversible. Specifically, their

kindling wavelength of said kindling stimulus ranges from about 200 to 1500 nm, their kindling stimulus ranges from about 0.01 to about 106 W/cm2, and their kindling duration of said kindling stimulus ranges from about 1 ms to about 60 min. The subject protein/nucleic acid compns. find use in labeling protocols, e.g., in labeling proteins, organelles, cells and organisms, etc., in a variety of different types of applications. Also provided are systems and kits for use in practicing such applications. The use of a KFP to study cell migration during embryogenesis, and to study migration of a mitochondrion are demonstrated.

AN 2002:663560 TOXCENTER
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DN CA13803021184W
TI Kindling fluorescent proteins from Anthozoa and Heteractis crispa and their mutants and methods for their use
AU Lukyanov, Sergey Anatolievich; Chudakov, Dmitry; Lukyanov, Konstantin
CS ASSIGNEE: Clontech Laboratories, Inc.
PI WO 2002096924 A1 5 Dec 2002
SO (2002) PCT Int. Appl., 96 pp.
CODEN: PIXXD2.
CY UNITED STATES
DT Patent
FS CAPLUS
OS CAPLUS 2002:927445
LA English
ED Entered STN: 20021224
Last Updated on STN: 20040622

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